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06/27/01

PATENT APPLICATION
Docket No.:2820.1000-000

1644

P#10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Jan E. Schnitzer and Philip Oh		
Application No.:	09/208,195	Group:	1644
Filed:	December 9, 1998	Examiner:	P. Nolan
For:	IMMUNOISOLATION OF CAVEOLAE		

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231

on 6/27/01 (Date) Christina M. McSweeney (Signature)

Christina M. McSweeney
Typed or printed name of person signing certificate

TRANSMITTAL OF DECLARATION
UNDER 37 C.F.R §1.131 OF JAN E. SCHNITZER, M.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please find enclosed herewith a copy of an executed Declaration Under 37 C.F.R.§1.131 of Jan E. Schnitzer, M.D. for filing in the captioned application. An un-executed copy was filed with a Request for Continued Examination on June 6, 2001.

Please charge any deficiency or credit any overpayment in the fees that may be due in this matter to Deposit Account No. 08-0380. A copy of this letter is enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

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Lexington, MA 02421-4799
Date: June 27, 2001

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DMH/RWM/cmm
June 6, 2001

PATENT APPLICATION
Attorney's Docket No.: 2820.1000-000
(BIDMC98-20)

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on 6/6/01 (Date) _____ (Signature)
Christina M. McSweeney
(Typed or printed name of person signing certificate)

DECLARATION UNDER 37 C.F.R. §1.131
OF JAN E. SCHNITZER, M.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Jan E. Schnitzer, of 1475 Trabert Ranch Road, Encinitas, California 92024, hereby declare and state that:

1. I am a named inventor on the above-reference patent application. I have reviewed the application and the claims as amended in the Amendment accompanying this Declaration prior to executing the Declaration.

2. I understand that the Examiner has rejected the claims of the referenced patent application as being anticipated by Stan, R.-V. *et al.* (*Molecular Biology of the Cell*, Vol 8:595-605 (1997), herein referred to as "Stan *et al.*"), stating that Stan *et al.* describe subjecting fractions of plasma membranes to immunoisolation by polyclonal antibodies which would inherently bind the oligomerized form of caveolae since they recognized the caveolae in its natural state, prior to being denatured. I have reviewed Stan *et al.* prior to executing this Declaration.

3. Stan *et al.* describe a method of purification of caveolae which includes immunoisolation of caveolae on anti-caveolin coated magnetic beads (Figure 2 of Stan *et al.*), using an antibody they had prepared themselves (see Stan *et al.*, page 598, second column, under the heading, "Antibody Characterization," where it is stated that "Our antibody was used exclusively for immunoisolation of caveolae on magnetic beads..."). This antibody, described as "anticaveolin-N" antibody, was separated from polyclonal sera raised in rabbits against synthetic peptide (anticaveolin-N peptide) covalently coupled to keyhole limpet hemocyanin. "Anticaveolin-N" antibody was thus a fraction of a polyclonal antiserum, and not a monoclonal antibody as is set forth in the claims of the application. A polyclonal antiserum, even one that has been affinity purified, does not have the same specificity as a monoclonal antibody: polyclonal antisera are subject to contamination, and may contain antibodies to different epitopes. Furthermore, Stan *et al.* do not provide characterization information regarding the anticaveolin-N antibody fraction: there is no demonstration of immunoprecipitation or of a binding curve, as is common in the art when describing an antibody. The validation of the anticaveolin-N antibody fraction, described in Figure 1 of Stan *et al.*, shows only a small part of the filter: it is possible that the anticaveolin-N antibody fraction may interact with other bands not shown in Figure 1. In addition, it can be seen from Figure 1 that a high amount of peptide is needed (1000 ng/ml, lane7) for complete inhibition of the antibody, and that unexpectedly, the irrelevant peptide also competes for interaction (see, e.g., lanes 6 and 7): these results indicate poor affinity and specificity of the anticaveolin-N antibody fraction.

4. The low affinity and specificity of the anticaveolin-N antibody fraction is due, at least in part, to the peptide used to prepare the fraction. Stan *et al.* state that the synthetic peptide

used for preparation of the anticaveolin-N antibody fraction used the N-terminal residues 1-14 of chicken caveolin (the caveolin-N peptide; see Stan *et al.*, page 597, first column, under the heading, "Antibody Production"). However, the 16 N-terminal residues set forth by Stan *et al.* (MSGGKYVSDSEGHLYC) are not the N-terminal residues of chicken caveolin; they differ from the N-terminal residues of chicken caveolin that are in the GenBank data base, as provided in the copies of each of the two entries pertaining to chicken caveolin that were submitted previously as Appendix I to the Amendment filed on October 3, 2000, in this application. They also do not match the N-terminal sequence of caveolin from any other species, as known to date. At most, 11 of the 16 residues set forth by Stan *et al.* are present together in the N-terminal region of chicken caveolin. One of ordinary skill in the art, recognizing that the synthetic peptide is 11/16 (less than 70%) identical to the N-terminal residues found in caveolin, would expect that an antibody which recognizes the synthetic peptide, such as the antibodies in the anticaveolin-N antibody fraction of Stan *et al.*, would likely have poor affinity to caveolin itself, and may react with other proteins.

5. In contrast to the anticaveolin-N antibody fraction of Stan *et al.*, which recognizes a synthetic peptide that is not identical to any peptide present in caveolin, the representative antibody described in the application (monoclonal antibody 2234, referred to in the application as CAV) specifically binds the alpha-isoform of caveolin-1 via a specific epitope found in the N-terminal segment that is not present in the beta-isoform (p. 13, lines 13-18, citing Scherer *et al.*, *J. Biol. Chem.* 270:16395-16401 (1995)) Details regarding the specific binding of CAV can be found, for example, in Oh, P. and Schnitzer, J.E., *Journal of Biological Chemistry* 274(33):23144-23154 (1999), referred to herein as "Oh and Schnitzer"). Oh and Schnitzer provide descriptions of experiments common in the art that are used to describe a new antibody: Western analysis for showing antibody monospecificity for caveolin, specifically its alpha-isoform (see, e.g., Fig. 1 of Oh and Schnitzer), and standard kinetic analysis of antibody binding to caveolin in its native state in caveolae of plasma membranes (see, e.g., Fig. 2 of Oh and Schnitzer).

6. It is most unlikely that the N-terminal residues coupled to the keyhole limpet hemocyanin, as used by Stan *et al.*, would have been able to form an epitope that is

present in the oligomerized caveolin structural cage that surrounds caveolae, particularly since the residues do not, in fact, match the N-terminal residues of chicken caveolin. The binding of the anticaveolin-N antibody fraction to the N-terminal peptide used by Stan *et al.*, is not equivalent to binding of an antibody to the oligomerized form of caveolae. One of ordinary skill in the art would not assume that an antibody which binds to caveolin would inherently also bind to caveolin in a native state as an oligomeric structure. In fact, as discussed in Oh and Schnitzer, several antibodies to caveolin were tested and only CAV had reactivity with caveolin in its native state as an oligomeric structure. The nature of the anticaveolin-N antibody fraction, especially with regard to caveolin, is rendered unclear by the dissimilarity of the immunogen (caveolin-N peptide) to caveolin. One skill in the art would not expect specific nor avid interaction with caveolin, and there is no reason to believe that it binds avidly to native, oligomerized caveolin that is present in the oligomerized caveolin structural cage that surrounds caveolae. Rather, one of ordinary skill in the art, given the Stan *et al.* reference, would assume at most, and with considerable hesitation, that the anticaveolin-N antibody fraction used by Stan *et al.* would bind to caveolin in the same manner as the commercial polyclonal antiserum (pAb) also used by Stan *et al.*, as pAb was used to monitor experimental samples of caveolae immunoisolated using the anticaveolin-N antibody fraction. It is likely, however, that such a commercial antibody would have a greater affinity, avidity, and specificity (e.g., to be reasonably monospecific for caveolin) than the anticaveolin-N antibody fraction of Stan *et al.* However, the commercial antibody did not bind avidly to caveolin oligomerized around intact caveolae (see, e.g., the antibody pAb describe in Oh and Schnitzer).

7. In contrast to the anticaveolin-N antibody fraction of Stan *et al.*, the representative antibody described in the application (CAV) not only specifically binds the alpha-isoform of caveolin-1 via a specific epitope found in the N-terminal segment that is not present in the beta-isoform, but also interacts with oligomeric caveolin on intact caveolae (see, e.g., Oh, P. and Schnitzer, *supra*). Oh and Schnitzer describe an inability of publicly available antibodies, including the polyclonal rabbit antiserum to caveolae (pAb) obtained from the same source as the pAb used in Stan *et al.*, to react effectively with oligomerized caveolin on intact caveolae, and the ability of monoclonal antibody clone 2234 (CAV) to caveolin

on intact caveolae (see Oh and Schnitzer, p. 23146, second column, "Characterization of an Antibody Reactive with Caveolin Cage of Caveolae," and Figure 1).

8. Although the anticavcolin-N antibody fraction described by Stan *et al.* did recognize caveolin, as indicated by the reduction and abrogation of the 22 kDa caveolin signal when antigen (N terminal peptide having the specified residues) was added to a sample of antibody and caveolin or antibody and P1 fraction (see Stan *et al.*, page 598, second column, under "Antibody Characterization"), the affinity of the anticaveolin-N antibody to caveolin was low, as indicated by the need for overnight incubation to obtain caveolae (see Stan *et al.*, page 597, second column). In sharp contrast, the representative antibody of the invention, CAV, bound extremely rapidly (e.g., within one hour) and with high affinity to caveolin, as demonstrated in the application (see Example 2 and Figure 2, which depicts the rapidity of CAV binding in contrast with that of other antibodies including the rabbit polyclonal antiserum pAb). These results are reiterated in the Oh and Schnitzer reference (see Oh and Schnitzer, page 23146-7, "Characterization of an Antibody Reactive with Caveolin Cage of Caveolae," particularly Figure 2).

9. In summary, the Stan *et al.* anticaveolin-N antibody fraction differs from the antibody used in the claimed invention in several aspects. For example, the Stan *et al.* anticaveolin-N antibody fraction is a polyclonal antiserum, whereas the antibody used in the claimed invention is a monoclonal antibody. In addition, the Stan *et al.* anticaveolin-N antibody fraction recognizes a synthetic peptide that is not identical to any peptide present in caveolin, whereas the antibody used in the claimed invention is specific for caveolin, as shown by the ability of the representative antibody CAV to bind to the alpha-isoform of caveolin-1 via a specific epitope found in the N-terminal segment. Furthermore, the Stan *et al.* anticaveolin-N antibody fraction does not appear to be able to bind to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae: Stan *et al.* suggest that the anticaveolin-N antibody fraction has an equivalent binding ability as the polyclonal antiserum pAb, and this polyclonal antiserum pAb does not bind to oligomerized caveolin in its native state as an oligomeric structural cage surrounding intact caveolae, as indicated by Oh and Schnitzer.

I further declare that all statements made herein of my knowledge are true and that all statements made on other information and belief are believed to be true; and further, that these

statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Jan E. Schnitzer

6/6/01

Date